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AGRICULTURAL EXTENSION SERVICE
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DETERMINATION OF ACID DEGREE VALUE BY A MODIFIED METHOD OF THOMAS, NIELSEN, AND OLSON[1]

EQUIPMENT

1. Babcock test centrifuge (unheated).
2. Standard 18-gm. 8% Babcock milk test bottle; or 9-gram 20% Babcock ice cream test bottle.
3. Water bath for maintaining test bottles at boiling temperature. Equip with rack or tray to hold test bottles.
4. Water bath to hold test bottles at 130° - 140° F. for tempering fat column.
5. 50-ml. graduated syringe with No. 15 needle; or a standard 17.6 ml. milk pipette.
6. 1.0-ml. tuberculin type syringe with No. 19 needle (4").
7. 50-ml. Erlenmeyer flask.
8. 5-ml. microburette.

REAGENTS

1. BDI Reagent. Thirty-gm. of Triton X-100* (a non-ionic surface-active agent) and 70 gm. of sodium tetraphosphate** are made up to 1 liter with distilled water. (Sodium hexametaphosphate, if uncaked, may be substituted for the sodium tetraphosphate if desired.)

   *Triton X-100 may be obtained from the Rohm and Haas Company, Philadelphia, Pa.
   **Sodium tetraphosphate may be obtained from the Rumford Division, Heyden Chemical Corporation, Rumford 16, Rhode Island.

2. Alcoholic KOH. Prepare 0.02 N KOH solution using absolute methanol (commercially pure grade). This solution should be standardized against standard potassium acid phthalate or other suitable standard.
3. Indicator solution. This is prepared by dissolving 1 gm. phenolphthalein in 100 ml. of absolute methanol.

[1]The original method was published under the title "Hydrolytic Rancidity in Milk - a Simplified Method for Estimating the Extent of Its Development," E. L. Thomas, A. J. Nielsen, and J. C. Olson, Jr., American Milk Review, January. 1955.

4. Aqueous methyl alcohol. This consists of equal volumes of absolute methanol (commercially pure grade) and distilled water. This mixture should be prepared with both reagents at room temperature.

5. Fat solvent: 4 parts petroleum ether (Skellysolve B) and 1 part N-propanol (reagent grade). This may be conveniently prepared by mixing 800 ml. of Skellysolve B with 200 ml. of N-propanol (commercially pure).

PROCEDURE

1. Place 35 ml. of milk in either type test bottle described above using either the 50-ml. syringe or the 17.6-ml. milk pipette. (Use of the 9-gm. 20% Babcock test bottle may better facilitate removal of the fat than use of the 18-gm. 8% bottle.)

2. Add 10 ml. of BDI reagent and mix thoroughly.

3. Place bottle in gently boiling water bath. Agitate contents of the bottle thoroughly after 5 and 10 minutes. Caution: After the last shaking, the bottles should remain in the boiling water bath a minimum of 5 minutes to effect a clear fat separation.

4. After a total of 15 to 20 minutes in the boiling water bath, remove and centrifuge for 1 minute.

5. Add sufficient aqueous methyl alcohol to bring the fat column well within the graduated portion of the neck of the test bottle.

6. Centrifuge for an additional 1 minute.

7. Place bottle in tempering water bath for 5 minutes. Water level of bath should be at or above top of fat column.

8. Transfer 1.0 ml. (or approximately 1.0 ml.) of the tempered fat from the test bottle to a 50-ml. Erlenmeyer flask using the 1.0-ml. syringe. Record the exact volume of fat transferrred. If exactly 1.0 ml. of fat can be removed, a 1.0 ml. volumetric pipette may be used. If a volumetric pipette is used, rinse with two 1.0-ml. portions of fat solvent.

9. Dissolve fat in 5 ml. of fat solvent. If volumetric pipette is used in step 8, reduce amount of solvent by the amount used to rinse the pipette (2.0 ml.). Add 5 drops of indicator solution.

10. Titrate to first definite color change with the standardized alcoholic KOH solution using the 5-ml. microburette. Occasionally turbidity in the solvent-fat mixture will be observed during titration. If this occurs, the addition of 2 or 3 ml. additional solvent should clear the mixture.

11. Express results in terms of acid degree value (ml. of 1N base required to titrate 100 gm. of fat). Method of calculation is described below.

NOTE: A blank titration should be made on the fat solvent (in the absence of fat) using 5 drops of the indicator. Blank titrations should be run on each new batch of solvent and these retitrated at frequent intervals thereafter.

CALCULATIONS OF ACID DEGREE VALUE (ADV)

1. Calculate the grams of milk fat in the sample titrated by multiplying the ml. of milk fat by the density of the milk fat after tempering (0.90).

   Grams of milk fat = ml. of milk fat x 0.90

2. Calculate the Acid Degree Value as follows:

$$ADV = \frac{(Ml.\ of\ KOH\ used\ in\ titration - Blank) \times N.\ F. \times 100}{Wt.\ of\ fat}$$

   N. F. = normality of alcoholic KOH solution

   Blank = titration value of the fat solvent in the absence of fat and using 5 drops of phenolphthalein as indicator. Blank determinations should be run on each new batch of solvent and then re-titrated at frequent intervals thereafter.

   Example: Assume that 0.75 ml. of fat required 0.70 ml. of alcoholic KOH having a normality of 0.0203 and the blank titration value was 0.03, then:

$$ADV = \frac{(0.70 - 0.03) \times 0.0203 \times 100}{0.075 \times 0.90} = \frac{0.67 \times 0.0203 \times 100}{0.675} = \frac{1.36}{0.675} = 2.014$$

   (ADV should be expressed to the second decimal place only in reporting the result, such as 2.01 for the above example.)

PROCEDURE FOR DETERMINING A.D.V. OF BUTTER

1. Melt butter in a container immersed in warm water.
2. Pipette 9 ml. of melted fat into a cream test bottle.
3. Add 5 ml. of BDI
4. Place in gently boiling water bath and hold 10 minutes, mixing contents after each 5 minute interval.
5. Add water to bring mixture up to the neck of the bottle.
6. Centrifuge 1 minute.
7. Place in gently boiling water bath for 5 minutes.
8. Add 50% aqueous methyl alcohol to bring fat well into the graduated neck of the test bottle.
9. Centrifuge 1 minute.
10. Carry out procedure from step 8 of A.D.V. test on whole milk.

PROCEDURE FOR DETERMINING A.D.V. OF CREAM

1. Determine the fat content of the cream to be tested by the Babcock or BDI detergent method.

2. Standardize the cream to 10% fat with water (weigh the cream and pipette the water).

3. Pipette 30 ml. of the standardized mixture (water and cream) into a Babcock milk test bottle and add 10 ml. of BDI detergent. Continue on through the regular procedure for determining the acid degree in whole milk.

NOTE: The A.D.V. of cream is not comparable to the value obtained on whole milk, i.e., the rancid flavor is readily noted at lower A.D.V.'s in cream than whole milk. Rancidity is detectable at an A.D.V. of 0.80 - 0.90 in cream, but is not readily noted until A.D.V.'s of 1.20 - 1.50 in whole milk.

NOTE: A test kit containing all necessary items for running A.D.V. determinations can be purchased from:

Meyer Blanke
30 Russell Blvd.
St. Louis 4, Missouri